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14. ABSTRACT
Two approaches to control microbiologically influenced corrosion (MIC) have ben developed that do not require the use of biocides. These strategies include the following: i) use of biofilms to inhibit or prevent corrosion, and ii)manipulation (removal or addition) of an electron acceptor, (e.g. oxygen, sulphate or nitrate) to influence the microbial population. In both approaches the composition of the microbial community is affected by small perturbations in the environment (e.g. temperature, nutrient concentration and flow) and the response of microorganisms cannot be predicted with certainty. The following sections will review the literature on the effectiveness of these environmentally friendly, "green," strategies for controlling MIC.

15. SUBJECT TERMS
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A review of 'green' strategies to prevent or mitigate microbiologically influenced corrosion

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Abstract

Two approaches to control microbiologically influenced corrosion (MIC) have been developed that do not require the use of biocides. These strategies include the following: i) use of biofilms to inhibit or prevent corrosion, and ii) manipulation (removal or addition) of an electron acceptor, (e.g. oxygen, sulphate or nitrate) to influence the microbial population. In both approaches the composition of the microbial community is affected by small perturbations in the environment (e.g. temperature, nutrient concentration and flow) and the response of microorganisms cannot be predicted with certainty. The following sections will review the literature on the effectiveness of these environmentally friendly, "green," strategies for controlling MIC.

Keywords: *Microbiologically influenced corrosion, biofilms, inhibition, electron acceptors*

Introduction

The traditional approach to treating/controlling microbiologically influenced corrosion (MIC) has been to use oxidising (e.g. chlorine, bromine, ozone) or non-oxidising (e.g. glutaraldehyde, carbamates, guanides, isothiazolines) biocides to reduce the numbers and types of organisms in a bulk medium. The problems with this approach are well documented. In some cases oxidising biocides can cause corrosion. Neither oxidizing nor non-oxidizing biocides penetrate biofilms. Costerton et al. (1994) reported that bacteria in biofilms were resistant to antibiotics and biocides at levels 500 to 5000 times higher than those required to kill planktonic cells of the same species. Persistent use of a single biocide treatment can allow more resistant microorganisms to develop and remain in the biofilm. Ridgway et al. (1984) demonstrated that bacteria previously exposed to chlorine were more resistant than those never exposed. Resistance to a particular biocide can be overcome by periodically changing the biocide. Numerous investigators have observed a rapid resumption of biofouling after a biocide treatment or mechanical removal of cells from a surface. Regrowth or recovery may be due to the following: i) Remaining viable cells reproducing a biofilm, ii) residual biofilm imparting a surface roughness that enhances transport and sorption, or iii) oxidation of extracellular polymeric substances and lysed cells may provide nutrients for regrowth.

Videla et al. (2004) described three "environmentally friendly" approaches to preventing bacterial attachment as the first step in preventing MIC: i) a natural biocide from the black mustard *Brassia nigra*, ii) film-forming mixtures of amines, imidazoline and quaternary ammonium compounds and iii) immunoglobulin A (IgA). However, there are potential problems with the three proposed approaches. The authors report that the biocidal efficacy of the natural biocide was slightly less when used on established biofilms and that current use of IgA is limited to medicinal applications. Alkyl imidazoline is a registered pesticide (US EPA) and is only registered for use in closed systems. Furthermore, some amines are toxic to animals. Videla et al. (2004) reported that all three approaches were effective in reducing microbial populations or inhibiting bacterial adherence. None of these approaches were evaluated for long-term effects or their direct effect on corrosion. Enzien et al. (1996) demonstrated that a film-forming quaternary amine inhibited microbial attachment under conditions found in oil and gas production. However, they cautioned that film-forming inhibitors

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can initiate localised corrosion if film coverage is not uniform.

Within the past few years, two approaches to control of MIC have been developed that do not require the use of biocides. These strategies include the following: i) use of biofilms to prevent corrosion, and ii) manipulation (removal or addition) of an electron acceptor to influence the microbial population.

Corrosion inhibition by biofilms

Corrosion inhibition due to the presence and activities of bacteria within biofilms has been reported for carbon steel (Pedersen & Hermansson, 1989; 1991; Hernandez et al. 1994; Jayaraman et al. 1997; 1999a) stainless steel (Jayaraman et al. 1999a) aluminum 2024 (Jayaraman et al. 1999b; Örnek et al. 2002; Zuo et al. 2005), and copper (Jayaraman et al. 1999b). The mechanisms most frequently cited for corrosion inhibition by biofilms are as follows: i) The biofilm forms a diffusion barrier to corrosion products that stifles metal dissolution, ii) respiring aerobic microorganisms within the biofilm consume oxygen, decreasing the concentration of that reactant at the metal surface, iii) microorganisms produce metabolic products that act as corrosion inhibitors (e.g. siderophores), iv) microorganisms produce specific antibiotics that prevent the proliferation of corrosion-causing organisms (e.g. sulphate-reducing bacteria [SRB]).

Several investigators have demonstrated that aerobic bacteria in a biofilm decrease the rate of corrosion of mild steel. Hernandez et al. (1994) observed increased polarisation resistance (R_p) when mild steel was exposed to a synthetic seawater medium (nine-salt solution [NSS]) containing *Pseudomonas* sp. S9 or *Serratia marcescens*. The NSS contained 17.6 g sodium chloride, 1.47 g sodium sulfate, 0.08 g sodium bicarbonate, 0.25 g potassium chloride, 0.04 g potassium bromide, 1.87 g hydrated magnesium chloride, 0.41 g hydrated calcium chloride, 0.008 g hydrated strontium chloride, 0.008 g bromic acid in 1 l ultrapure water. They measured a marked increase in R_p values for the exposures in the presence of either bacterium (Table I). Surface biofilms were stained with acridine orange and imaged with epifluorescence microscopy. Higher R_p values for surfaces colonised by *S. marcescens* compared to those colonised by *Pseudomonas* sp. S9 were attributed to higher numbers of *S. marcescens* cells attached to the metal surface. Electrochemical impedance spectroscopy (EIS) spectra (Figure 1) after immersion in sterile and *Pseudomonas* sp. inoculated NSS demonstrated the same basic trends indicated by the R_p data. The phase angle vs. frequency showed a maximum at 45° for sterile NSS, indicating Warburg-type impedance and a diffusion-controlled reaction. In contrast, the maximum phase angle in bacterial suspensions was 75° and the modulus vs. frequency indicated little corrosion during the first 20 days.

Table I. R_p of mild steel (KΩ-cm^2) after different exposure times to NSS with bacteria (4×10^8 cells ml^{-1})[A] and without (sterile) (Hernandez et al. 1994 © NACE International 1994).

	Exposure Time (days)		
	10	20	30
Pseudomonas sp. S9	28 ± 3	[B]	[B]
S. marcescens	35 ± 5	34 ± 3	15 ± 2
Sterile NSS	5.0 ± 0.5	4 ± 0.5	3.5 ± 0.5

[A]Results correspond to a mean value obtained on 5 specimens ± the standard deviation.
[B]The system was contaminated after 20 days.

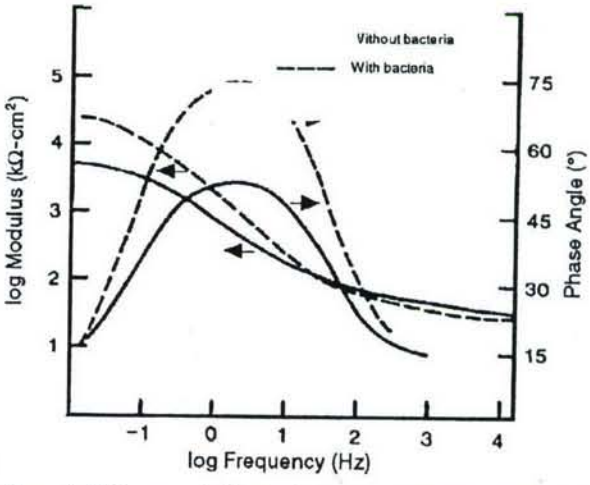

Figure 1. EIS spectra obtained after 20 days of exposure with and without *Pseudomonas* sp. S9. Curves are given for individual runs. Standard deviation between replicates was ± 10% (Hernandez et al. 1994 © NACE International 1994).

In addition, Hernandez et al. (1994) concluded the following: i) corrosion inhibition required bacterial adhesion; ii) the inhibition effect disappeared when *in situ* cells were fixed in glutaraldehyde; iii) when cell-covered carbon steel surfaces were transferred to nutrient-deficient synthetic seawater, the inhibition continued despite the predicted diminished respiration; iv) after exposure to natural seawater the inhibitive effect disappeared and *Pseudomonas* could not be located in the biofilm.

Jayaraman et al. (1997) designed experiments to investigate whether or not corrosion inhibition by aerobic biofilms was a general phenomenon. They used carbon steel (SAE 1018) coupons exposed in a complex liquid medium (Luria-Bertani [LB] broth) and an augmented synthetic seawater Vaatanen nine-salt solution (VNSS) and 15 different pure-culture

bacterial suspensions representing seven genera. Surface biofilms were stained with a viability assay kit and imaged with a confocal microscope. Compared to sterile controls, the mass loss in the presence of bacteria decreased by 2- to 15-fold (see Table II). Corrosion inhibition varied among the genera and the extent of corrosion inhibition depended on the nature of the biofilm: an increased proportion of live cells decreased corrosion. The authors concluded that a thin layer of attached actively respiring cells was required to inhibit corrosion.

Eashwar and Maruthamuthu (1995) reviewed the literature on ennoblement (a positive shift in corrosion potential [E_{corr}]) of metals in marine environments. They concluded that ennoblement is regulated by metabolic activity rather than the physical presence of microorganisms in a biofilm and hypothesised that a strengthening of the passive film on stainless steel alloys is siderophore-assisted. Siderophores are iron-chelators formed by bacteria at near neutral pH. Others have reported corrosion inhibition properties of siderophores (McCafferty & McArdle, 1992). Eashwar and Maruthamuthu (1995) rationalised the range of ennoblement that has been reported in the literature as due to alloy-siderophore compatibility, i.e. "an intrinsic property of the alloy to profit from the presence of the inhibitor".

Jayaraman et al. (1999a) used modified *Bacillus subtilis* in a complex, nutrient-rich medium (modified Baar's) to produce antimicrobial peptides, including indolicidin and bactenecin, to inhibit growth of SRB. Modified and unmodified *B. subtilis* biofilms grown on 304 stainless steels were exposed to SRB. EIS was used to characterise the corrosion behavior through measurement of the Rp. *In situ* production of bactenecin (3.0–3.5 $\mu g\ ml^{-1}$) by modified *B. subtilis* within the biofilm inhibited growth of SRB and resulted in a 12-fold reduction in the corrosion rate in comparison to unmodified biofilms. Örnek et al. (2002) demonstrated that pitting attack of aluminum 2024 in LB medium was reduced by the anionic peptides, polyaspartate and polyglutamate secreted by genetically engineered and natural *B. subtilis* and *B. licheniformis*, respectively. Formation of an aluminum/polyaspartate or polyglutamate complex may have reduced the uniform corrosion rate of aluminum.

Despite the laboratory studies indicating possibilities for using bacteria to inhibit corrosion of a number of alloys, applications have not been totally successful. Arps et al. (2003) evaluated the concept of corrosion control using regenerative biofilms at Three Mile Island (TMI) Nuclear Power Station, Harrisburg, PA. The field experiment was conducted using a consortium of five bacteria (one polymyxin-producing strain, *Paenibacillus polymyxa* 10401, and four gramicidin S producing bacillus strains) to inoculate service water in a side stream containing coupons of 304 stainless steel, 1018 carbon steel, and cartridge brass under different flow rates. Their results indicate that the inhibition of corrosion, measured by reciprocal polarisation resistance ($1/R_p$, instantaneous corrosion rate), by the consortium was small (Figure 2). Lower flow rates resulted in higher E_{corr} values. Pitting of brass specimens was noted in the presence or absence of the consortium (Figure 3). Both pit densities and pit areas were lower for samples exposed to the tailored consortium or to the single organisms *P. polymyxa* 10401. Neither $1/R_p$ nor pit area/density provide information as to the extent of localised corrosion.

Table II. Bacterial strains, antibiotic resistances, and corrosion ($mg\ cm^{-2}$) of SAE 1018 steel coupons after 1 week in LB medium or VNSS medium with 15 aerobic bacteria at 30°C. Data represent the average of three coupons and standard deviations are included (adapted from Jayaraman et al. 1997, and reprinted with permission from Springer-Verlag).

Bacterium	Corrosion in LB ($mg\ cm^{-2}$)	Corrosion in VNSS ($mg\ cm^{-2}$)	Antibiotic resistance ($\mu g\ ml^{-1}$)
Sterile LB Medium	1.03 ± 0.14	1.37 ± 0.15	–
Streptomyces lividans TK23.1	0.51 ± 0.08	0.24 ± 0.03	thiostrepton (50)
Bacillus subtilis	0.39 ± 0.06	did not grow	–
Bacillus circulans	0.26 ± 0.06	0.37 ± 0.05	–
Rhizobium meliloti 102F34	0.18 ± 0.06	0.17 ± 0.02	
Pseudomonas fragi K	0.17 ± 0.02	0.52 ± 0.08	kanamycin (100)
Escherichia coli BK6	0.16 ± 0.04	0.45 ± 0.08	tetracycline (25)
Bacillus brevis	0.14 ± 0.04	0.19 ± 0.07	–
Burkholderia cepacia G4	0.13 ± 0.04	0.22 ± 0.02	ampicillin (50)
Agrobacterium tumefaciens A114	0.13 ± 0.03	0.23 ± 0.05	–
Bacillus migulanus	0.11 ± 0.02	0.74 ± 0.07	–
Escherichia coli HB101/pRK2013	0.11 ± 0.02	0.41 ± 0.05	kanamycin (50)
Pseudomonas mendocina KR1	0.10 ± 0.01	0.14 ± 0.02	ampicillin (50)
Pseudomonas fluorescens 2-79	0.09 ± 0.01	0.36 ± 0.11	ampicillin (50)
Pseudomonas putida KT2440	0.09 ± 0.02	0.38 ± 0.05	ampicillin (50)
Pseudomonas putida F1	0.07 ± 0.01	0.46 ± 0.02	ampicillin (50)

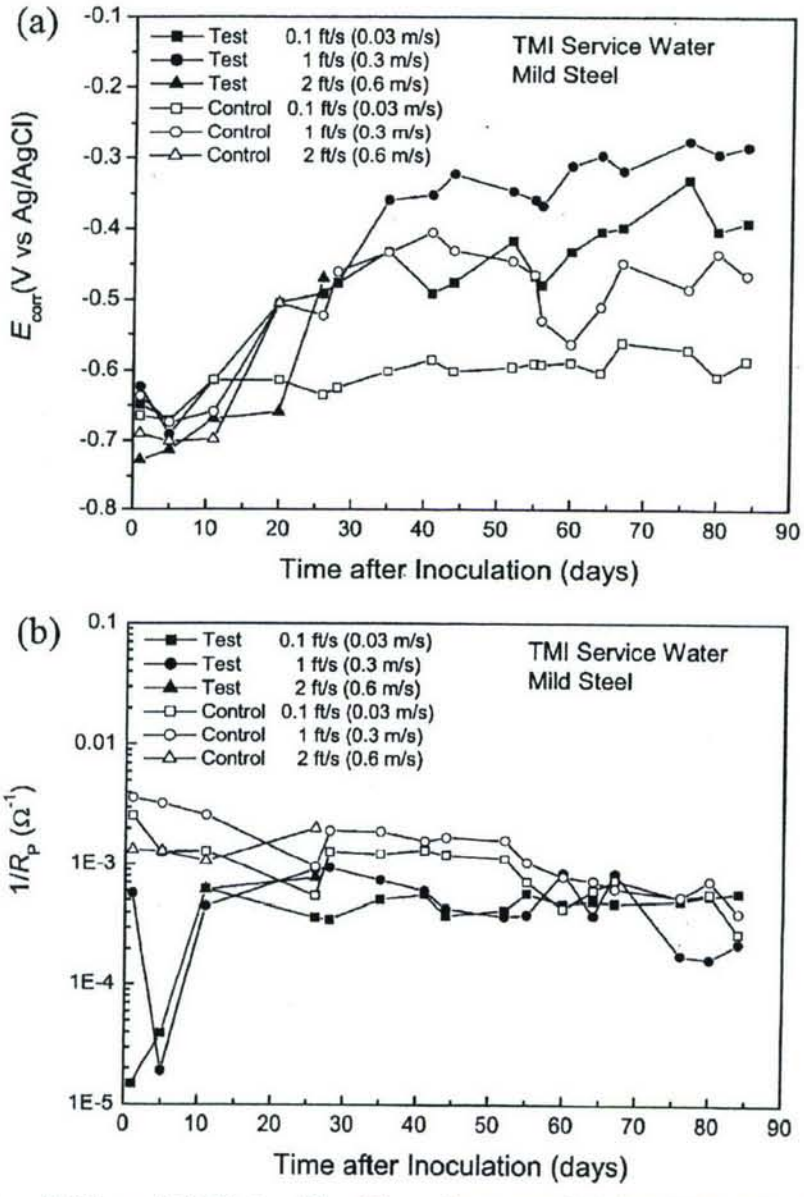

Figure 2. Time dependence of (a) E_{corr} and (b) $1/R_p$ for mild steel disc specimens exposed to a bacterial consortium containing five strains. The test (▲) and control (Δ) specimens at 60.96 cm (2 ft) s^{-1} were monitored for ~3 weeks before the monitoring cables were switched to brass specimens (Arps et al. 2002 © NACE International 2002).

Literature on corrosion inhibition by biofilm is confusing because the same organisms and mechanisms, which reportedly cause MIC, can also inhibited corrosion. For example, Pedersen et al. (1988) reported that *Pseudomonas* sp. S9 and *Serratia marcescens EF 190* caused an increase in the corrosion of iron and nickel compared to sterile conditions. They coated glass slides with a layer of iron and nickel and monitored corrosion as the appearance of transparent patches "at the site of contact between the metal and the bacterial colony". Their experiments were conducted on agar plates containing VNSS and Lewins Marine Medium (LMM) at 20°C. The same researchers have shown that *Pseudomonas* sp. S9 *and S. marcescens EF 190* can have a protective effect on carbon steel (ASTM A619) in VNSS at 18–20°C (Pedersen & Hermansson, 1989; 1991). Metal binding by extracellular polymers has been reported as a mechanism for both MIC (Geesey & Jang, 1988) and for corrosion inhibition (Ford et al. 1988).

Jigletsova et al. (2004) and Rodin et al. (2005) examined the influence of environmental conditions on corrosion inhibition by biofilms and demonstrated that the division of bacteria into ones that caused corrosion and ones that inhibited corrosion

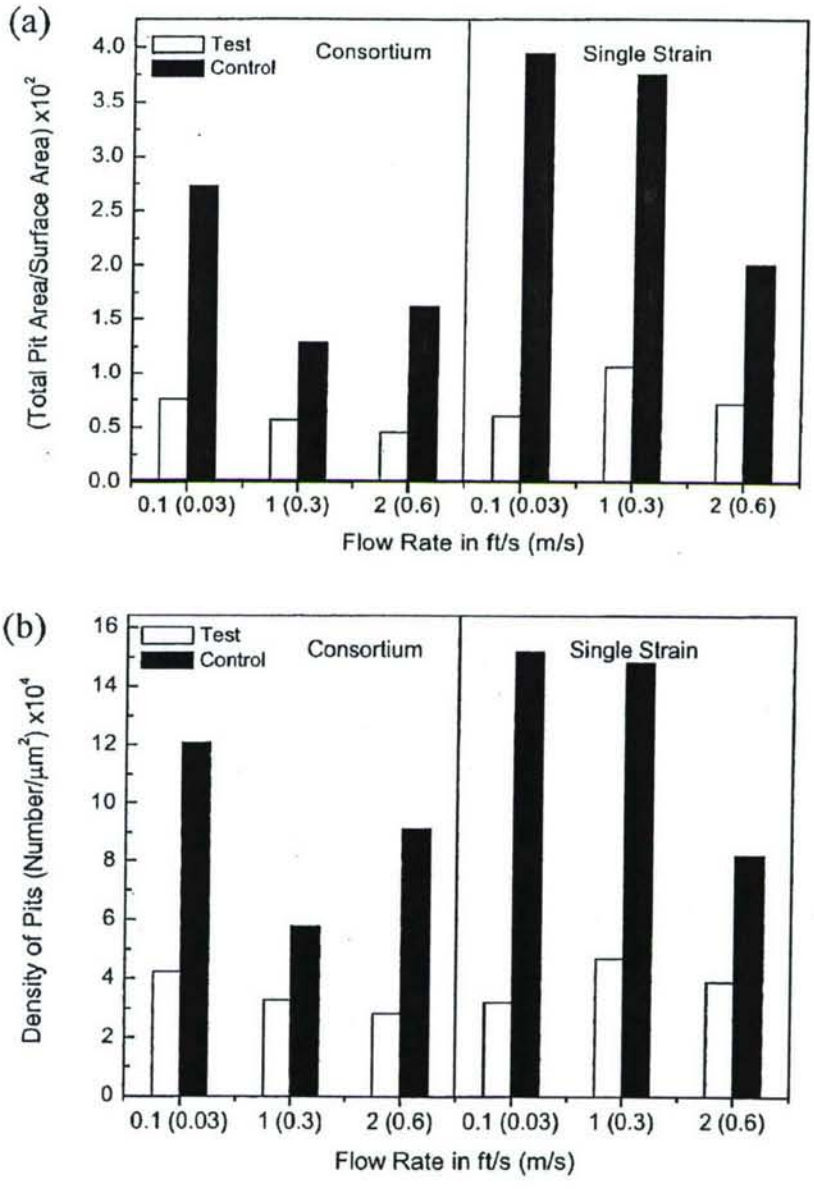

Figure 3. Comparative pitting results of cartridge brass specimens exposed to TMI service water in the bacterial consortium and single strain experiments: (a) relative pit area, and (b) density of pits (Arps et al. 2002 © NACE International 2002).

was entirely arbitrary and that the corrosive properties of biofilms varied with culture conditions, especially with culture medium composition. Rodin et al. (2005) used mild steel coupons exposed to a natural consortium of bacteria, including oil-oxidising aerobes and SRB isolated from oil-processing waters. An increase in corrosion was observed when coupons were transferred from LB to a glucose minimal medium with peptone (GMP) (Figure 4a). During biofilm formation in GMP, corrosion increased vs. sterile control. However, corrosion decreased when coupons with biofilms were transferred into enriched LB medium (Figure 4b). Their data indicate that environmental conditions determine the specific microbiological effect on corrosion processes – not the individual organisms. Dubiel et al. (2002) used EIS to evaluate corrosion inhibition in the presence of iron-reducing bacteria. They concluded that the physiology of the bacteria in the biofilm, the flow rate and the chemistry of the electrolyte determine the ultimate impact of microorganisms on corrosion.

Webster and Newman (1994) also examined the impact of media constituents on localised corrosion of an Fe-15Cr-10Ni stainless steel and observed that localised corrosion would not readily occur unless chloride ion was the predominant anion in the medium. They concluded that chloride must be

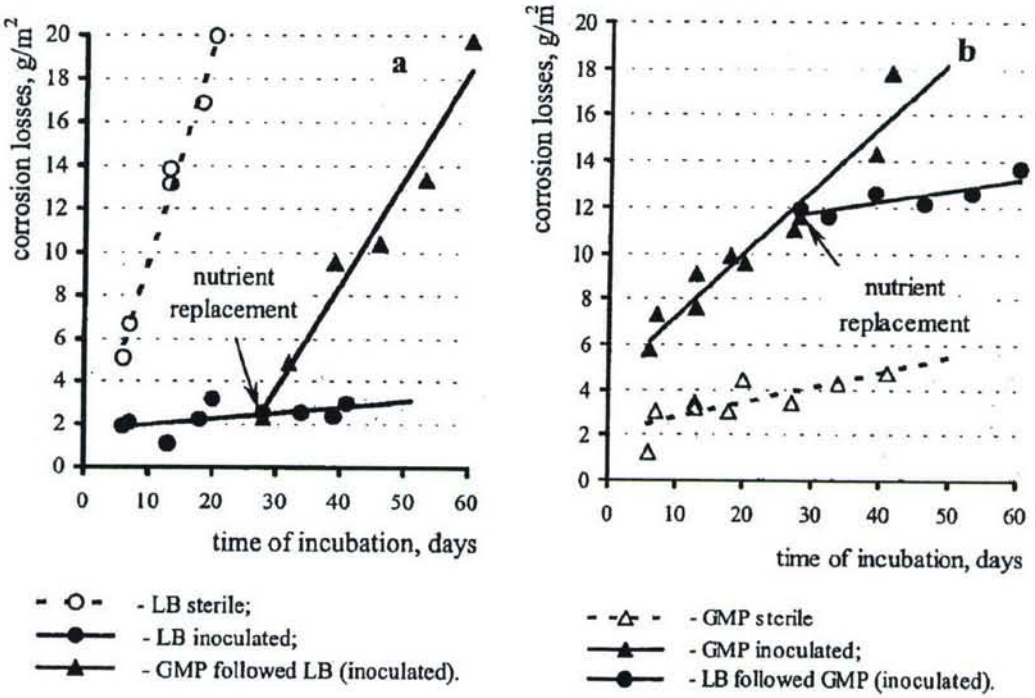

Figure 4. The dynamics of corrosion losses with nutrient replacement: (a) The biofilm grown in LB was transferred into GMP; (b) the biofilm grown in GMP was transferred into LB medium (Rodin et al. 2005 © NACE International 2005).

present in a concentration at least comparable to that of all other anions combined, otherwise corrosion was inhibited even at high H_2S concentrations up to 500 ppm. Reduction of the ratio of Cl^- ions to other anions increased the time to initiation and decreased the rate of propagation of the corrosion. Other corrosion investigators have concluded that extra nutrients cannot be added to stimulate bacterial growth if those nutrients inhibit corrosion by adding too many non-chloride ions (Ringas & Robinson, 1988). Anions (e.g. sulfate, hydroxide, phosphate, acetate, carbonate and nitrate) can inhibit pitting corrosion. It is possible that bacterial consumption and fixation of nutrients, including sulfate could render an initially inhibiting solution aggressive by removing non-chloride ions.

An additional complication in the interpretation of the laboratory studies on biofilm inhibition of corrosion is the effect of culture media on electrochemical measurements and on corrosion reactions. Most of the laboratory studies on corrosion inhibition have been conducted in laboratories with nutrient-rich media. The impact of nutrients on electrochemical measurements and MIC was investigated by Webster and Newman (1994). They observed interferences in electrochemical measurements when yeast extract was included in the culture medium/electrolyte. The interferences were removed when the yeast extract was removed. Many of the nutrient-rich media used in the cited laboratory studies on corrosion inhibition contained yeast extract. Modified Baar's medium contains 10 g l^{-1} tryptone, 5 g l^{-1} yeast extract, and 10 g l^{-1} NaCl. The LB medium contains 10 g l^{-1} tryptone, 5 g l^{-1} yeast extracts and 10 g l^{-1} NaCl. VNSS consists of 1.0 g peptone, 0.5 g glucose, 0.5 g starch, 0.5 g yeast extract, 0.01 g hydrated ferrous sulfate, 0.01 g $NaHPO_4$ and 1,000 ml of NSS. Studies on corrosion inhibition in complex media have compared corrosion in sterile and inoculated media, but none have carefully studied the impact of media constituents on the electrochemical parameters used to quantify corrosion.

Alter potential electron acceptors to inhibit specific groups of bacteria

Both removal and addition of electron acceptors has been used as a means of controlling microbial populations and MIC in seawater injection systems where seawater is injected into oil reservoirs to maintain pressure. In these applications, oxygen is removed to minimise corrosion. However, in the anaerobic environment, growth of SRB is encouraged and corrosion of iron and steel alloys is the result. The concentration of sulfate in seawater is >2.0 g l^{-1}. Rizk et al. (1998) used nanofiltration to reduce sulfate in seawater from 2.6 g l^{-1} to 50 mg l^{-1}. In laboratory studies they were able to

demonstrate that the amount of hydrogen sulfide was a direct function of the amount of sulfate in the water. The authors discussed the implication for corrosion, but did not make corrosion measurements. In contrast, Jhobalia et al. (2005) demonstrated that high sulfate concentration in the medium (increases from 1.93 g l^{-1} to 6.5 g l^{-1}) could inhibit growth of *Desulfovibrio desulfuricans* and the corrosion rate of mild steel (Figure 5a & b). Corrosion was directly related to numbers of SRB in the bulk medium. Cells were counted using a haecytometer. The authors hypothesised that the observation was due to increasing toxicity of sulfate towards SRB metabolism or sulfate reduction.

Laboratory and field experiments have demonstrated that nitrate treatment can be an effective alternative to biocide treatment to reduce the numbers of SRB and their activity, a process known as biocompetitive exclusion. The addition of nitrate can induce a shift in the dominant population from SRB to nitrate-reducing bacteria (NRB). Nitrate treatment was implemented on an oil platform in the North Sea (Veslefrikk) (Thorstenson et al. 2002). The change from glutaraldehyde treatment to nitrate resulted in a substantial change in the bacterial community. The SRB population decreased and the numbers of NRB increased (Figure 6). After 4 months of nitrate addition the activity of SRB in the

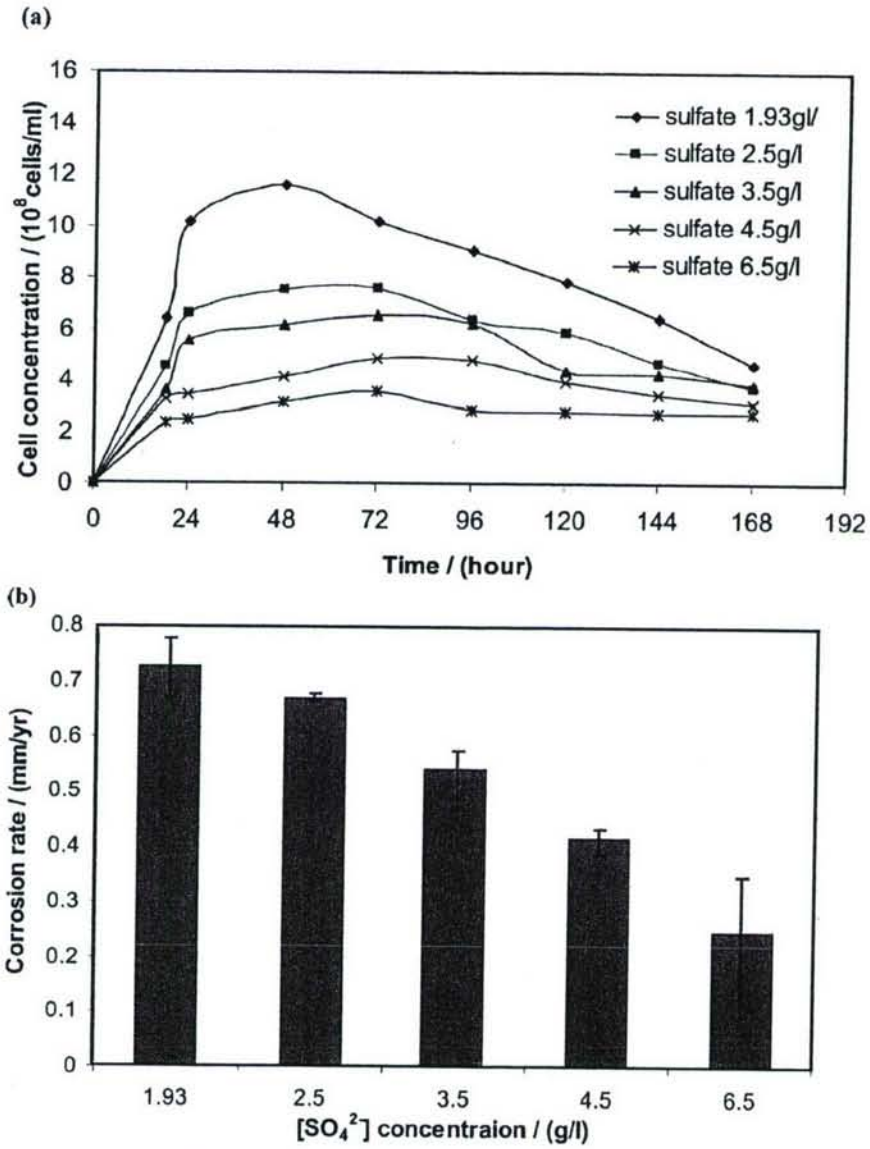

Figure 5. (a) SRB growth with time at different initial sulfate concentrations in anaerobic vials (Jhobalia et al. 2005 © NACE International 2005). (b) Corrosion rate (weight loss) at different initial sulfate concentrations in the medium after inoculation at 37°C. Weight loss was measured at the end of 7 days (Jhobalia et al. 2005 © NACE International 2005).

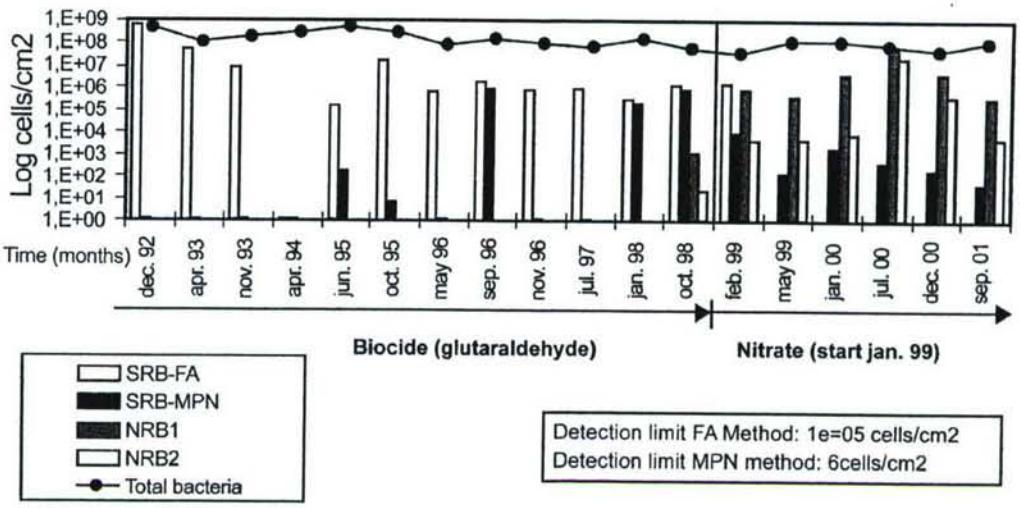

Figure 6. Number of bacteria in biofilm using two detection techniques: fluorescent antibody (FA) and most probable number (MPN). Column marked NRB1 shows number of cells in MPN series targeting facultatively anaerobic NRB, and column marked NRB2 shows number of cells in MPN series targeting obligatory anaerobic NRB (Thorstenson et al. 2002 © NACE International 2002).

biofilm was markedly reduced as measured with respiratory methods and an enrichment of NRB was measured. After 32 months' nitrate treatment, SRB numbers were reduced 20,000-fold and SRB activity was reduced 50-fold. Corrosion measurements decreased from 0.7 mm $year^{-1}$ to 0.2 mm $year^{-1}$. Similar applications have been made to reduce souring (Larsen, 2002; Larsen et al. 2004). Gullfaks platforms (Sunde et al. 2004) have been treated with nitrate to reduce H_2S production. A 1000-fold reduction in SRB numbers and a 10 to 20-fold reduction in sulfate respiration activity and a 50% reduction in corrosion. The authors suggest that reservoir characteristics and nutrient availability have a significant impact on the effectiveness of nitrate injection.

Voordouw et al. (2002) and Hubert et al. (2006) demonstrated a nitrate-reducing, sulfide oxidising bacterium capable of reducing nitrate to nitrite, nitrous oxide or nitrogen and oxidising sulfide to sulfate or sulfur. The stoichiometry of the reactions catalysed by the organism depends on the ratio of sulfide to nitrate. Dunsmore et al. (2004) isolated an organism from a Danish North Sea oilfield water injection system that had been continuously treated with nitrate since the start of the injection. This species, a SRB, could reduce nitrate and produce ammonium in the presence of sulfate, increasing the likelihood of corrosion.

Hubert et al. (2004) demonstrated that both nitrate and nitrite are effective treatments for decreasing sulfide concentrations (Table III). The required dose depended on the concentration of oil organics used as the energy source by the microbial community. Because of its higher oxidative power, nitrate can remove more oxidisable oil organics than nitrite. However, nitrite is a strong inhibitor of SRB.

Table III. Weight loss of coupons buried in the sand matrix of bioreactors in response to nitrate treatment (Hubert et al. 2004 © NACE International 2004).

Treatment	Weight loss (%)[1]
None	8.3 ± 5.3
Nitrate (17.5 mM)	4.7 ± 1.3
Nitrite (20 mM)	0.7 ± 0.2
Control[2]	0.8 ± 0.1

[1]Average for 15 corrosion coupons ($\pm$SD). Total incubation time ~110 d.
[2]Incubated in the absence of nitrate or nitrite for 45 days (batchwise and increasing flow rate periods only).

The success of nitrate addition relies on a population of nitrate-utilising bacteria in the system. Hubert et al. (2006) suggested that bioaugmentation, in which *ex situ* grown microorganisms could be injected with the nitrate if indigenous NRB were lacking. Despite the possibility of bioaugmentation, there are several reports of failures. Bouchez et al. (2000) attempted to inoculate a nitrifying sequencing batch reactor with an aerobic denitrifying bacterium. The added bacterium disappeared after two days. Similarly, Hubert et al. (2004) reported that introduction of microorganisms into natural communities is difficult.

Both the addition and removal of oxygen have been proposed as corrosion control measures. Khanal and Huang (2003) demonstrated that oxygenation was effective in controlling sulfides during anaerobic treatment of high-sulfate wastewater.

However, SRB in biofilms depend on other organisms to remove oxygen and produce nutrients, so they can survive in aerated systems. Furthermore, oxygen exacerbates the problem of corrosion. Hamilton (2003) proposed a model for MIC in which he concluded that several MIC mechanisms involved a process of electron transfers from base metal to oxygen as the ultimate electron acceptor through a series of coupled reactions. The specific coupled reactions varied with mechanism and causative organism. In the case of SRB, sulfate, an intermediate electron acceptor, is reduced to sulfide that reacts with a metal to form a corrosion product that ultimately transfers electrons to oxygen. Consistent with that model, most reported cases of SRB induced corrosion are in environments with some dissolved oxygen in the bulk medium (Hardy & Bown, 1984; Lee et al. 1993).

Removing oxygen from seawater has been proposed as a corrosion control measure for unprotected carbon steel ballast tanks. Matsuda et al. (1999) conducted shipboard trials by sealing a ballast tank at the deck and installing vertical pipes into the headspace. They reported that pumping pure nitrogen gas into the headspace for 1.5 h reduced oxygen levels in the seawater to approximately 0.2 mg l^{-1} and decreased the rate of uniform corrosion of carbon steel by 90% as determined by weight loss. However, in laboratory experiments, Lee et al. (2004) compared corrosion of 1020 carbon steel resulting from stagnant aerobic natural seawater with corrosion resulting from stagnant anaerobic natural seawater over a one-year period (Figure 7). They demonstrated the following: i) corrosion was more aggressive under totally anaerobic conditions as measured by $1/R_p$ and weight loss, ii) under aerobic conditions corrosion was uniform and the surface was covered with iron oxides (lepidocrocite and goethite), iii) under anaerobic conditions the corrosion was localised pitting and the corrosion products were mackinawite and pyrrothite, and iv) under anaerobic conditions $1/R_p$ depended on coupon position where rows 1–4 were positioned from top to bottom. Lee et al. (2005) designed field experiment to evaluate deoxygenation of natural seawater as a corrosion control measure for unprotected carbon steel seawater ballast tanks. They demonstrated the difficulty of maintaining hypoxic seawater. Using a gas mixture it was possible to displace dissolved oxygen. However, aerobic respiration and corrosion reactions consumed oxygen and produced totally anaerobic conditions within the first days of hypoxia. When gaskets and seals failed, oxygen was inadvertently introduced. The impact of oxygen ingress on corrosion depends on the amount of oxygen in the system at the time oxygen is introduced. Carbon steel exposed to cycles of hypoxic seawater and oxygenated atmosphere had higher corrosion rates than coupons exposed to cycles of either consistently aerobic or deoxygenated conditions. Technologies are commercially available for the deoxygenation of seawater for ballast tanks. There are no data on the long-term benefits of these treatments on corrosion inhibition.

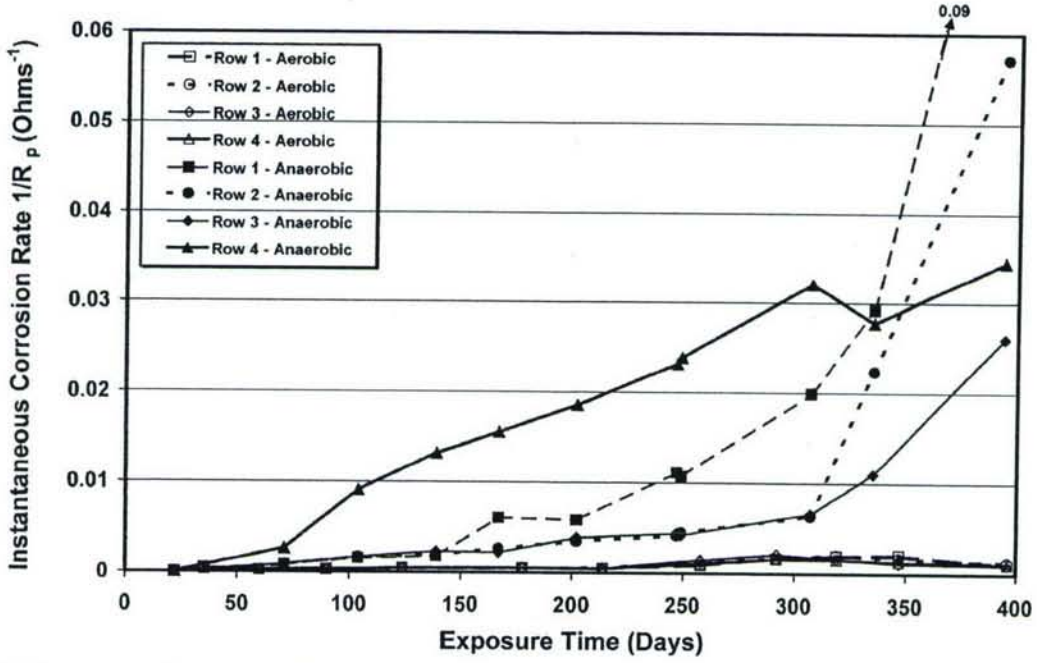

Figure 7. In laboratory experiments using 1020 carbon steel, the corrosion resulting from stagnant aerobic natural seawater was compared with corrosion resulting from stagnant anaerobic conditions (Lee et al. 2004).

Discussion

It is difficult to compare the laboratory studies on corrosion inhibition due to biofilms because of the differing experimental conditions, organisms and culture conditions. The following critical issues must be addressed before bacteria can be used to predictably inhibit corrosion: i) The stochastic nature of biofilms, and ii) contamination and/or natural competition. One of the fundamental assumptions in much of the work on corrosion inhibition by biofilms is that biofilm formation is predictable and controllable. Microorganisms colonise all engineering materials, but there is a stochastic nature to areal coverage and thickness that has never been successfully modeled. Bacteria in pure cultures or in consortia do not form uniform, predictable biofilms. Growth rate depends on substratum, available nutrients, temperature and electron acceptors. Cells within biofilms die and can cause aggressive corrosion. Clumps of cells can slough from the surface transforming a homogeneous biofilm to a patchy one. Furthermore, biofilm composition is affected by small perturbations in the environment (e.g. temperature, nutrient concentration, and flow). The response of microorganisms within biofilms cannot be predicted with certainty. Natural competition by extraneous organisms can alter the microbial constituents of a biofilm. Hernandez et al. (1994) observed contamination of controls after brief experiments (20 days) and a change in microbial composition of biofilms after introduction into a natural system. The bigger problem is that most investigators do not evaluate the microbial population at the end of the experiment and have no insight into possible contamination or changes in the engineered biofilm.

Nitrate/nitrite supplementation is a new technology and some work is underway to carefully characterise the bacteria that are developing in nitrate-rich water. NRB reduce nitrate to N_2 with several possible intermediates, including nitrite. There are several potential mechanisms for the observed inhibition of SRB due to addition of nitrate. One of these is competition for carbon sources. When competing for the same carbon source, NRB out-compete SRB because nitrate is a stronger oxidiser than sulfate. This argument is valid only in carbon-limited waters. Toxic reaction products from the reduction of nitrate to N_2 may inhibit SRB. A shift in the redox potential in the system may also inhibit SRB. As a consequence of nitrate reduction, the redox potential will likely increase, producing unfavorable conditions for sulfate reduction.

The conflicting results between the corrosion experiments of Matsuda et al. (1999) and Lee et al. (2004) using deoxygenation may be due to method of dissolved oxygen removal. Matsuda et al. (1999) used bubbled pure nitrogen gas, while Lee et al. (2004) used an anaerobic hood that contained a mixture of nitrogen, carbon dioxide, and hydrogen gases. As discussed by Lee et al. (2007), bubbling nitrogen gas into seawater not only displaced dissolved oxygen but also displaced dissolved carbon dioxide, which provides the buffering capacity of seawater. Displacement of dissolved carbon dioxide by pure nitrogen gas raised the pH of natural seawater from 8.0 to over 9.0, creating a less conducive environment for bacterial growth, particularly SRB. In comparison, seawater maintained in an anaerobic hood with mixed gases had a pH of between 6.5 and 7.0, with elevated levels of SRB and biotic sulphide.

Conclusions

Biocides control MIC by decreasing the microbial population, whereas control by manipulation of electron acceptor and by corrosion inhibiting biofilms relies on stimulation or retardation of specific microbial populations. Corrosion inhibition due to biofilms has been demonstrated in the laboratory for several microorganisms on several metals and alloys, but has never been demonstrated in a field application. Controlling electron acceptor (e.g. oxygen, sulfate and nitrate) concentration has been used successfully in limited commercial applications. All applications have been limited to seawater. Nitrate addition and sulfate removal/reduction both attempt to control MIC due to SRB. The long-term consequences on the microbial populations and MIC are unknown.

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References

Arps PJ, Xu LC, Green RM, Wood TK, Mansfeld FB, Syrett BC, Earthman JC. 2003. Field evaluation of corrosion control using regenerative biofilms (CCURB). CORROSION/2003. Houston, TX: NACE International. Paper No. 03714.

Bouchez T, Patureau D, Dabert P, Juretschko S, Dore J, Delgenes P, Molette R. 2000. Ecological study of a bioaugmentation failure. Environ Microbiol 2:179–190.

Costerton JW, Ellis B, Lam K, Johnson F, Khoury AE. 1994. Mechanisms of electrical enhancement of efficacy of antibiotics in killing biofilm bacteria. Antimicrobial Agents Chemother 38: 2803–2809.

Dubiel M, Hsu CH, Chien CC, Mansfeld F, Newman DK. 2002. Microbial iron respiration can protect steel from corrosion. Appl Environ Microbiol 68:1440–1445.

Dunsmore BC, Whitfield TB, Lawson PA, Collins MD. 2004. Corrosion by sulfate-reducing bacteria that utilize nitrate. CORROSION/2004. Houston, TX: NACE International. Paper No. 04763.

Eashwar M, Maruthamuthu S. 1995. Mechanisms of biologically produced ennoblement: ecological perspectives and a hypothetical model. Biofouling 8:203–213.

Enzien MV, Pope DH, Wu MM, Frank J. 1996. Nonbiocidal control of microbiologically influenced corrosion using organic film-forming inhibitors. CORROSION/1996. Houston, TX: NACE International. Paper No. 290.

Ford T, Maki JS, Mitchell R. 1988. Involvement of bacterial exopolymers in biodeterioration of metals. In: Houghton D, Smith RN, Eggins HO, editors. Biodeterioration 7. London/New York: Elsevier. pp 378–384.

Geesey G, Jang L. 1989. Binding of metal ions by extracellular polymer. In: Beveridge T, Doyle R, editors. Metals, ions and bacteria. New York: John Wiley and Sons. pp 325–357.

Hamilton WA. 2003. Microbiologically influenced corrosion as a model system for the study of metal microbe interactions: a unifying electron transfer hypothesis. Biofouling 19:65–76.

Hardy JA, Bown JL. 1984. The corrosion of mild steel by biogenic sulfide films exposed to air. Corrosion 42:650–654.

Hernandez G, Kucera V, Thierry D, Pedersen A, Hermansson M. 1994. Corrosion inhibition of steel by bacteria. Corrosion 50: 603–608.

Hubert C, Voordouw G, Nemati M, Jenneman G. 2004. Is souring and corrosion by sulfate-reducing bacteria in oil fields reduced more efficiently by nitrate or by nitrite? CORROSION/2004. Houston, TX: NACE International. Paper No. 04762.

Hubert C, Voordouw G, Arensdorf J, Jenneman G. 2006. Control of souring through a novel class of bacteria that oxidize sulfide as well as oil organics with nitrate. CORROSION/2006. Houston, TX: NACE International. Paper No. 06669.

Jayaraman A, Earthman JC, Wood YK. 1997. Corrosion inhibition by aerobic biofilms on SAE 1018 steel. Appl Microbiol Biotechnol 47:62–68.

Jayaraman A, Hallock PJ, Carson RM, Lee CC, Mansfeld FB, Wood TK. 1999a. Inhibiting sulfate-reducing bacteria in biofilms on steel with antimicrobial peptides generated in situ. Appl Microbiol Biotechnol 52:267–275.

Jayaraman A, Örnek D, Duarte DA, Lee CC, Mansfeld FB, Wood TK. 1999b. Axenic aerobic biofilms inhibit corrosion of copper and aluminum. Appl Microbiol Biotechnol 52:787–790.

Jhobalia CM, Hu A, Gu T, Nesic S. 2005. Biochemical engineering approaches to MIC. CORROSION/2005. Houston, TX: NACE International. Paper No. 05500.

Jigletsova SK, Rodin VB, Zhirkova NA, Alexandrova NV, Kholodenko VP. 2004. Influence of nutrient medium composition on the direction of microbiologically influenced corrosion. CORROSION/2004. Houston, TX: NACE International. Paper No. 04575.

Khanal SK, Huang J-C. 2003. ORB-based oxygenation for sulfide control in anaerobic treatment of high-sulfate wastewater. Water Res 37:2053–2062.

Larsen J. 2002. Downhole nitrate applications to control sulfate reducing bacteria activity and reservoir souring. CORROSION/2002. Houston, TX: NACE International. Paper No. 02025.

Larsen J, Malene RH, Zwolle S. 2004. Prevention of reservoir souring in the Halfdan field by nitrate injection. CORROSION/2004. Houston, TX: NACE International. Paper No. 04761.

Lee JS, Ray RI, Little BJ. 2007. Comparison of Key West and Persian Gulf seawaters. CORROSION//2007. Houston, TX: NACE International, Paper No. 07518.

Lee JS, Ray RI, Lemieux E, Little BJ. 2005. An evaluation of deoxygenation as a corrosion control measure for ballast tanks. J Corrosion 65:1173–1188.

Lee WC, Lewandowski Z, Okabe S, Characklis WG, Avci R. 1993. Corrosion of mild steel underneath aerobic biofilms containing sulfate-reducing bacteria. Part I: at low dissolved oxygen concentration. Biofouling 7:197–216.

Lee JS, Ray RI, Lemieux E, Falster A, Little BJ. 2004. An evaluation of carbon steel corrosion under stagnant seawater conditions. Biofouling 20:237–247.

Matsuda M, Kobayashi S, Miyuki H, Yosida S. 1999. An anticorrosion method for ballast tanks using nitrogen gas. Report of Research and Development to the Ship and Ocean Foundation, October 1999. Japan.

McCafferty E, McArdle JV. 1992. Corrosion inhibition by biological siderphore. In: Proc 182nd Society Meeting. Toronto: The Electrochemical Society. pp 185–186.

Örnek D, Jayaraman A, Syrett B, Hsu C-H, Wood, TK. 2002. Pitting corrosion inhibition of aluminum 2024 by *Bacillus* biofilms secreting polyaspartate or γ-polyglutamate. Appl Microbiol Biotechnol 58:651–657.

Pedersen A, Hermansson M. 1989. The effects on metal corrosion by *Serratia marcescens* and a *Pseudomonas sp.* Biofouling 1: 313–322.

Pedersen A, Hermansson M. 1991. Inhibition of metal corrosion by bacteria. Biofouling 3:1–11.

Pedersen A, Kjelleberg S, Hermansson M. 1988. A screening method for bacterial corrosion of metals. J Microbiol Methods 8:191–198.

Ridgway HF, Justice CA, Whittaker C, Argo DG, Olson BH. 1984. Biofilm fouling of RO membranes – its nature and effect on treatment of water for reuse. J Am Water Works Ass (AWWA) 76:94–102.

Ringas C, Robinson F. 1988. Corrosion of stainless steel by sulfate-reducing bacteria – electrochemical techniques. Corrosion 44:386–396.

Rizk TY, Stott JFD, Eden RD, Davis RA, McElhiney JE, Di Iorio C. 1998. The effects of desulphated seawater injection on microbiological hydrogen sulphide generation and implication for corrosion control. CORROSION/1998. Houston, TX: NACE International. Paper No. 298.

Rodin VB, Zhigletsova SK, Zhirkova NA, Alexandrova NV, Shtuchnaja GV, Chugunov VA, Kholodenko VP. 2005. Altering environmental composition as a potential method for reversing microbiologically influenced corrosion. CORROSION/2005. Houston, TX: NACE International. Paper No. 05498.

Sunde E, Lillebo B-L P, Bodtker G, Torsvik T, Thorstenson T. 2004. H_2S inhibition by nitrate injection on the Gullfaks field. CORROSION/2004. Houston, TX: NACE International. Paper No. 04760.

Thorstenson T, Bodtker G, Lillebo B-L P, Torsvik T, Sunde E, Beeder J. 2002. Biocide replacement by nitrate in seawater injection systems. CORROSION/2002. Houston, TX: NACE International. Paper No. 02033.

Videla VA, Guiamet PS, Gómez de Saravia S, Herrera LK, Gaylarde CC. 2004. Environmentally friendly approaches to inhibit biocorrosion – an overview. CORROSION/2004, Houston, TX: NACE International. Paper No. 04574.

Voordouw G, Nemati M, Jenneman GE. 2002. Use of nitrate-reducing, sulfide-oxidizing bacteria to reduce souring in oil fields: interactions with SRB and effects on corrosion. CORROSION/2002. Houston, TX: NACE International. Paper No. 02034.

Webster BJ, Newman RC. 1994. Producing rapid sulfate-reducing bacteria-influenced corrosion in the laboratory. In: Kearns J, Little BJ, editors. Microbiologically influenced corrosion testing. Philadelphia, PA: ASTM Publication STP 1232. pp 28–41.

Zuo R, Kus E, Mansfeld F, Wood TK. 2005. The importance of live biofilms in corrosion protection. Corrosion Sci 47: 279–287.